GENE CONTROL OF NON-MENDELIAN VARIEGATION IN *NICOTIANA TABACUM*[1]

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IN the great majority of reports involving non-Mendelian plastid inheritance, the data indicate that nuclear genes exert no influence on the inheritance of abnormal plastids. These plastid mutations are assumed to arise spontaneously and to be irreversible. The inheritance of this type of non-Mendelian chlorophyll abnormality has been summarized in comprehensive reviews by RENNER (1936) and CORRENS (1937). Plastids and plasmon elements which influence plastid phenotypes may mutate spontaneously, but at present rigorous proof of the existence of such spontaneous mutations is lacking.

The once facile assumption of spontaneous mutations in plastids has been made uncertain by the demonstration of gene-induced plastid mutations. Irreversible changes in plastids, induced by nuclear genes, have been described in Hordeum (IMAI 1936; ARANSON, HARRINGTON and FRIESEN 1946; ARANSON and WALKER 1949; HAGEMANN and SCHOLZ 1962), in Oryza (PAL and RAMANUJAM 1941), in Triticum (MATSUMURA, 1962), and in Zea (RHOADES 1943, 1946).

A leaf variegation, originating by mutation in the plasmon or plastom of *Nicotiana tabacum* L. var. Hicks Broadleaf, has been found to be influenced by nuclear genes. The inheritance of this leaf variegation is described in this report.

MATERIALS AND METHODS

The variegation described in this report originated in a field grown plant of the tobacco variety Hicks Broadleaf. The variegated plant was transplanted to a greenhouse where it was self and reciprocally cross pollinated with the (normal) *N. tabacum* varieties Hicks Broadleaf, Turkish, NN Turkish, Burley 21, Xanthi, Type 63, and Dixie Shade. All self and cross pollinations were made in a screened greenhouse with plants grown in pots containing steam-sterilized soil. Seedling populations, examined for segregation of variegated and normal green plants, were grown in a screened greenhouse in flats containing steam-sterilized soil. Variegated stocks were cleft-grafted with four normal scions of each of the varieties Turkish, NN Turkish, Florida 22, and Xanthi. The graft generation and first sexual generation after grafting were examined for graft-transmission of variegation.

RESULTS

Segregating populations consisted of varying numbers of variegated and green

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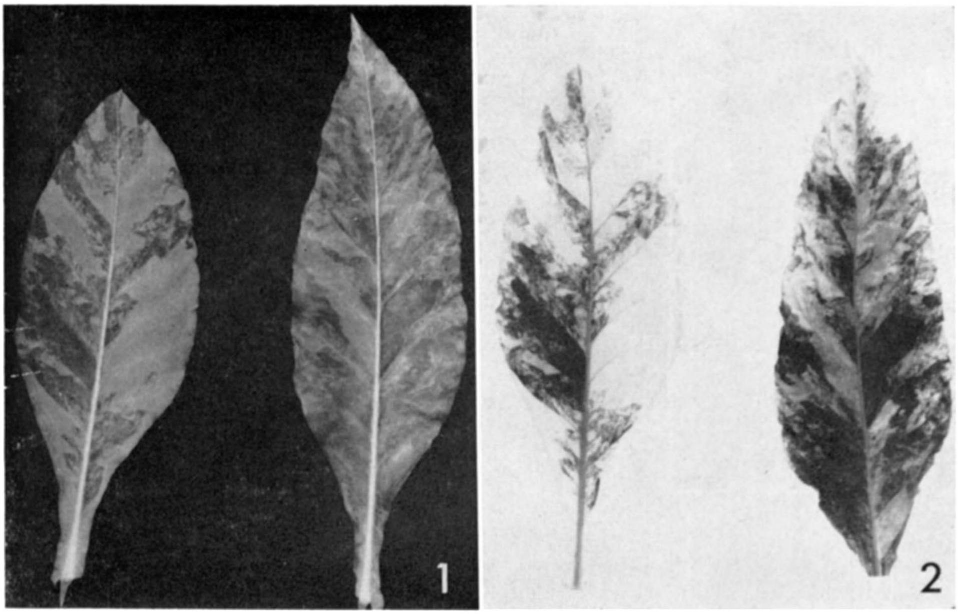

FIGURE 1.—Upper surfaces of variegated Hicks Broadleaf tobacco leaves.

FIGURE 2.—Upper surfaces of leaves shown in Figure 1 after removal of chlorophyll with ethanol, starch stained with IKI.

plants. Variegation was not expressed in the cotyledons but appeared in the first and subsequently developed leaves (Figure 1). Variegated leaves exhibited irregularly shaped green, pale green and yellow-green areas. The varying intensities of green observed in the pale and yellow-green areas are attributed to differences in thickness of cell layers containing normal and chlorotic plastids. Differences in starch accumulation shown in Figure 2 closely parallel the differences in intensity of greenness shown in Figure 1. None of the populations examined in the present study contained albino seedlings.

Variegation did not appear in any of the 16 normal scions grafted on variegated stocks. Three S_1 populations (1112 plants) derived from selfing flowers on normal scions of Turkish, NN Turkish, and Florida 22 varieties produced only green plants, and three S_1 populations (1982 plants) from normal scions of the Xanthi variety produced only green plants.

Frequencies of variegated and normal green seedlings obtained from self and cross pollinations of variegated and green plants are summarized in Tables 1 and 2. Self pollinations of green plants and crosses involving green plants as female parents produced populations containing only green plants. Self pollinations of variegated plants produced populations consisting of both green and variegated plants. The proportions of variegated and green plants varied greatly, with green plants usually predominating. When used as male parents in crosses on variegated plants, the varieties Hicks Broadleaf, Dixie Shade, Type 63, Burley 21, and Xanthi (Table 1) had no apparent influence on the expression or transmission

TABLE 1

Segregation of variegated and normal plants in populations derived from self and cross pollinations of tobacco varieties containing permitter genes

Parents	S_1 phenotype		Green S_1 selfed S_2 phenotypes		Variegated S_1 selfed S_2 phenotypes	
	Green	Variegated	Green	Variegated	Green	Variegated
Hicks O. V.* Capsule 1	284	24	602	0	505	21
Hicks O. V.* Capsule 2	707	289	751	0	210	43
	F_1 phenotypes		**Green F_1 selfed F_2 phenotypes**		**Variegated F_1 selfed F_2 phenotypes**	
	Green	Variegated	Green	Variegated	Green	Variegated
Hicks Variegated S_1 ♀ × Hicks normal S_1 ♂	456	227	602	0	144	165
Hicks Normal S_1 ♀ × Hicks Variegated S_1 ♂	609	0	528	0	...	...
Hicks O. V.* ♀ × Dixie Shade ♂	457	83	846	0	975	148
Dixie Shade ♀ × Hicks Variegated S_1 ♂	435	0	1219	0	...	...
Hicks Variegated S_1 ♀ × Type 63 ♂	531	119	817	0	466	73
Type 63 ♀ × Hicks Variegated S_1 ♂	1026	0	465	0	...	...
Hicks Variegated S_1 ♀ × Burley 21 ♂	207	37	1159	0	404	52
Burley 21 ♀ × Hicks Variegated S_1 ♂	134	0	332	0	...	...
Hicks Variegated S_1 ♀ × Xanthi ♂	423	94	645	0	587	17
Xanthi ♀ × Hicks Variegated S_1 ♂	634	0	762	0	...	...

* Hicks Broadleaf original variegated plant.

TABLE 2

Segregation of variegated and green plants in populations produced by crossing varieties containing permitter and eliminator genes

Parents	F_1 phenotypes		F_1 Green F_2 phenotypes	
	Green	Variegated	Green	Variegated
Hicks O. V.* ♀ × Turkish ♂	652	0	915	0
Turkish ♀ × Hicks O. V. ♂	1181	0	566	0
NN Turkish ♀ × Hicks Variegated S_1 ♂	328	0	819	0
Hicks O. V. ♀ × NN Turkish ♂	397	0	992	0
			BC_1 phenotypes	
			Green	Variegated
1. Hicks Variegated S_1 ♀ × (Hicks O. V. ♀ × Turkish ♂) F_1 ♂			331	37
2. (Hicks O. V. ♀ × Turkish ♂) F_1 ♀ × Hicks Variegated S_1 ♂			343	0
3. (Hicks O. V. ♀ × Turkish ♂) F_1 ♀ × Hicks Normal S_1 ♂			374	0
4. Hicks Variegated S_1 ♀ × (Hicks O. V. ♀ × NN Turkish ♂) F_1 ♂			406	23
5. (Hicks O. V. ♀ × NN Turkish ♂) F_1 ♀ × Hicks Variegated S_1 ♂			483	0
6. (Hicks O. V. ♀ × NN Turkish ♂) F_1 ♀ × Hicks Normal S_1 ♂			617	0

* Hicks O. V. signifies Hicks Broadleaf original variegated plant.

of variegation: When these varieties were used as female parents in crosses by variegated males, only green progeny were produced. Variegation was transmitted only by variegated plants through egg cells. Ratios of variegated and green plants did not fit any pattern permitting an explanation of inheritance based on nuclear gene segregation.

In contrast to the results summarized in Table 1, the varieties Turkish and NN Turkish suppressed variegation. Reciprocal crosses of Hicks Broadleaf variegated with Turkish and NN Turkish plants produced data which are summarized in Table 2. No variegated plants occurred in the progeny of these reciprocal crosses. Populations consisting of green and variegated plants (Backcross populations 1 and 4, Table 2) were derived from pollinating variegated plants with (Hicks variegated ♀ × Turkish ♂) F_1 male or (Hicks variegated ♀ × NN Turkish ♂) F_1 male. The populations derived from the reciprocals of these crosses contained only green plants. The Turkish and NN Turkish varieties prevent the expression and transmission of variegation.

DISCUSSION

In previous reports of non-Mendelian variegation in tobacco (Burk, Stewart and Dermen 1964; Burk and Grosso 1963; Dermen 1960; von Wettstein 1961; Wolf 1959) some albino seedlings were observed in the progeny of variegated plants. No albino plants occurred in the present study; this condition however is not unique since albino seedlings have not been reported in progenies of non-Mendelian variegated Humulus (Winge 1919), Plantago (Kappert 1953), or Borrago (Noack 1931).

It seems unlikely that pathogens are inducing agents of the leaf variegation since variegation did not occur in normal scions grafted on variegated stocks, and the progeny of selfed flowers from normal scions contained only green plants. The abnormal plastids which give rise to variegation may have originated by spontaneous plastid mutation. There is no evidence that the variegation originating in Hicks Broadleaf is influenced in any manner by inducer genes. However, the demonstration that inducer genes are not present in the Hicks Broadleaf material used in this study (Table 1, progenies of green F_1's selfed) does not exclude the possibility of gene-induced variegation. The variegation may have been gene-induced and subsequently associated with normal noninducer alleles long before its detection in the field.

The most frequently cited example of gene induced irreversible plastid mutation is that of *iojap* striping in *Zea mays* L. (Rhoades 1943, 1946). In this case some normal plastids were induced to mutate to forms reduced in size and lacking chlorophyll by homozygous recessive *ij ij* genes. Self pollinated *ij ij* homozygotes produced variable proportions of green, striped, and albino seedlings. Neither the heterozygous nor homozygous normal alleles influenced the phenotype of the mutant plastids. Thus it is possible to obtain striped (variegated) plants with abnormal plastids, originated by gene induced mutation, but with nuclei containing the noninducer *Ij Ij* alleles.

The proportions of variegated and normal green plants in the segregating populations in Tables 1 and 2 are not explicable on the basis of nuclear gene control. The progeny of normal green plants consisted entirely of green plants; variegation was transmitted only by variegated plants through egg cells. There is no evidence that the varieties listed in Table 1 when used as male parents exerted any influence on the expression or transmission of variegation. Therefore, the data in Table 1 are interpreted to indicate that variegation is controlled by extra-nuclear factors. The varieties Hicks Broadleaf, Dixie Shade, Burley 21, Type 63, and Xanthi are assumed to contain permitter genes; these genes are incapable of inducing or eliminating variegation, but rather they permit the expression and transmission of variegation.

With the exception of the present study and the reports of MAZOTI (1950, 1952, 1954), the existence of permitter genes has been assumed in all reported cases of non-Mendelian variegation, although this assumption has rarely, if ever, been explicitly stated. Populations produced by *ij ij* variegated females × multiple tester males have been found by MAZOTI (l.c.) to contain only green plants, or green and variegated plants, and no albino seedlings were observed. RHOADES (1956) stated that MAZOTI has demonstrated nuclear control of gene induced mutant plastids in corn.

The data in Table 2 do not support an assumption that permitter genes are present in either the Turkish or NN Turkish varieties. The data are interpreted to indicate that these varieties possess nuclear genes which block the expression and transmission of variegation. The existence of eliminator genes has not been reported in any previously recorded case of spontaneous non-Mendelian variegation. An assumption implicit in all previous descriptions of spontaneously occurring non-Mendelian variegation has been that the expression and transmission of variegation are independent of the genotype of the male parent.

There is no evidence in the present study that the variegation did not originate spontaneously. If the abnormal plastids in Hicks Broadleaf originated by spontaneous mutation, then the eliminators detected in Turkish and NN Turkish control a hitherto undescribed type of gene-cytoplasmic interaction. There is also no evidence that inducer genes are involved in the control of variegation, although the possibility remains that the variegated material originated by gene induction. If the variegation in Hicks Broadleaf originated by gene induced mutation of plastids, then the eliminators would be involved in gene-cytoplasmic interactions similar to but not identical to those described by MAZOTI.

The mechanism controlling elimination of variegation is not known. The suppression of variegation by the Turkish and NN Turkish varieties is complete, since variegation does not occur even in the presence of presumably homozygous permitter genes (Table 2, F_2 phenotypes and Backcrosses 2, 3, 5, 6). Therefore, the interaction of variegation eliminator genes with cytoplasmic factors is not analogous to the well known interaction of fertility restorer genes with cytoplasmic sterility factors. Previous assumptions concerning the independence of cytoplasmic factors in the control of leaf variegation are made questionable by the detection of eliminator genes reported in this study.

SUMMARY

Studies of a leaf variegation in the Hicks Broadleaf tobacco variety have shown no indication of pathogen induction of variegation and no evidence that inducer genes are involved in the inheritance of the variegation. The inheritance of the variegation is non-Mendelian with transmission only through egg cells. The varieties Hicks Broadleaf, Dixie Shade, Burley 21, Type 63, and Xanthi possess nuclear genes which permit the expression of variegation; the varieties Turkish and NN Turkish contain genes which eliminate the variegation.

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